

1/20

FIG. 1

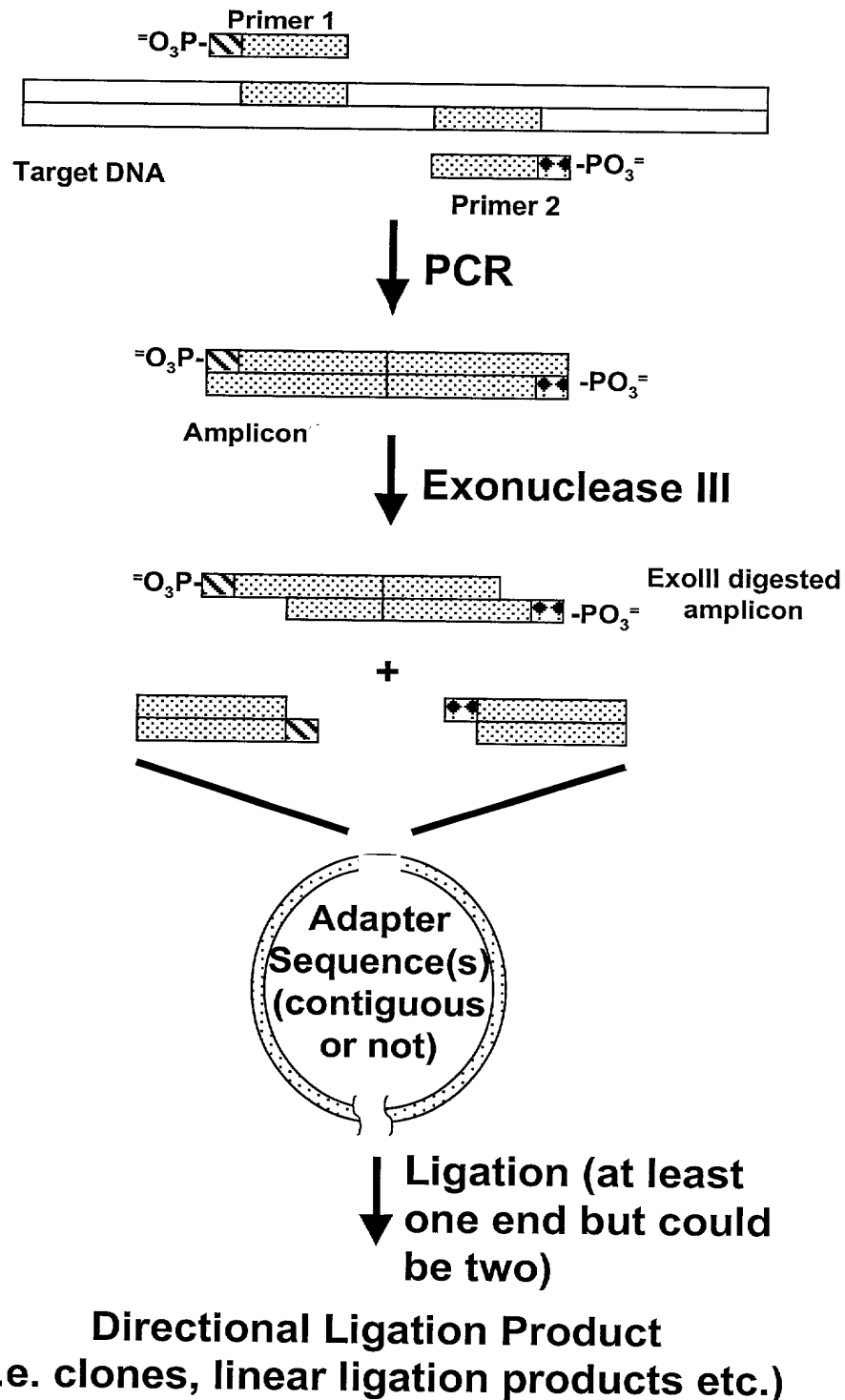


FIG. 2

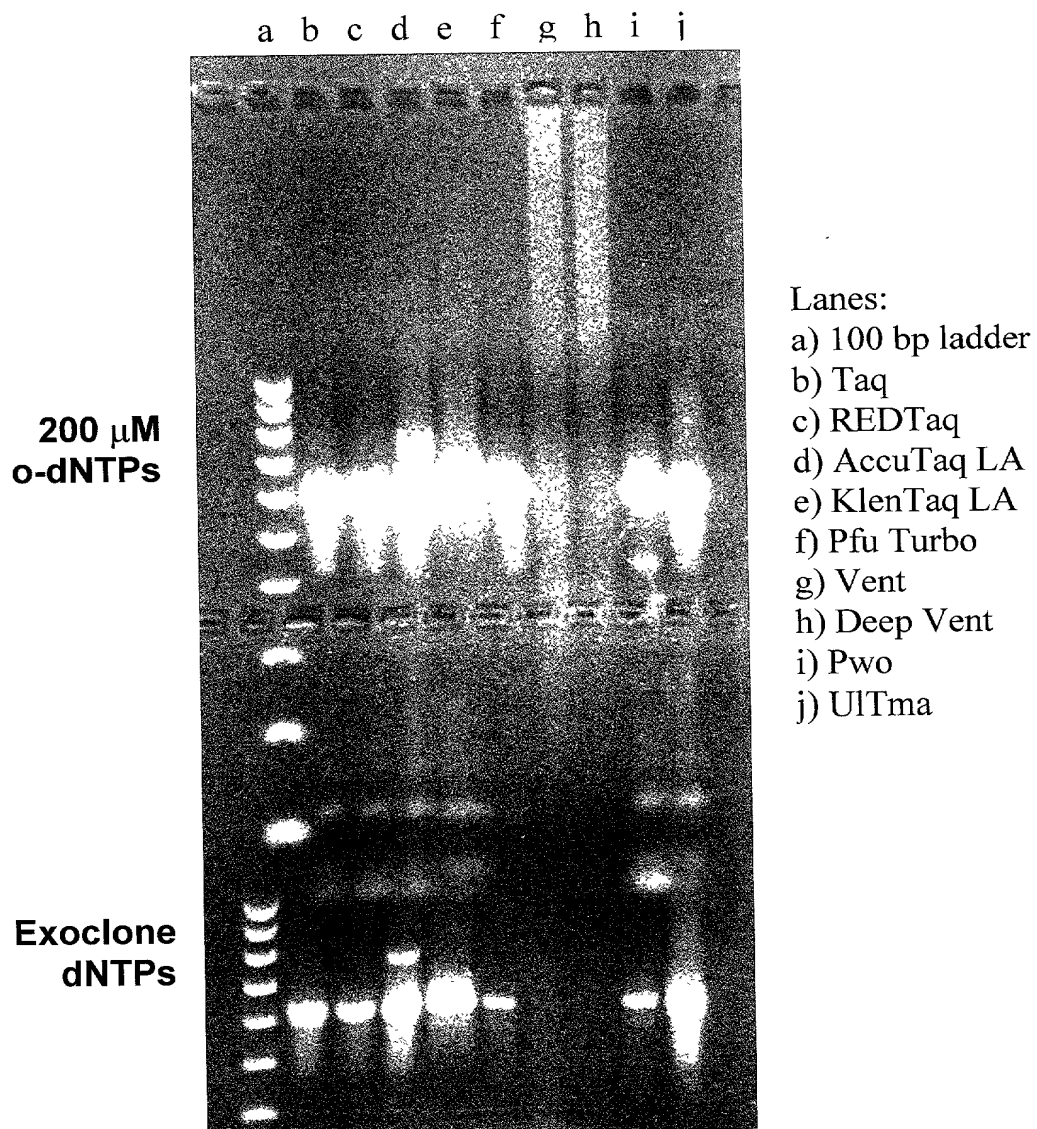
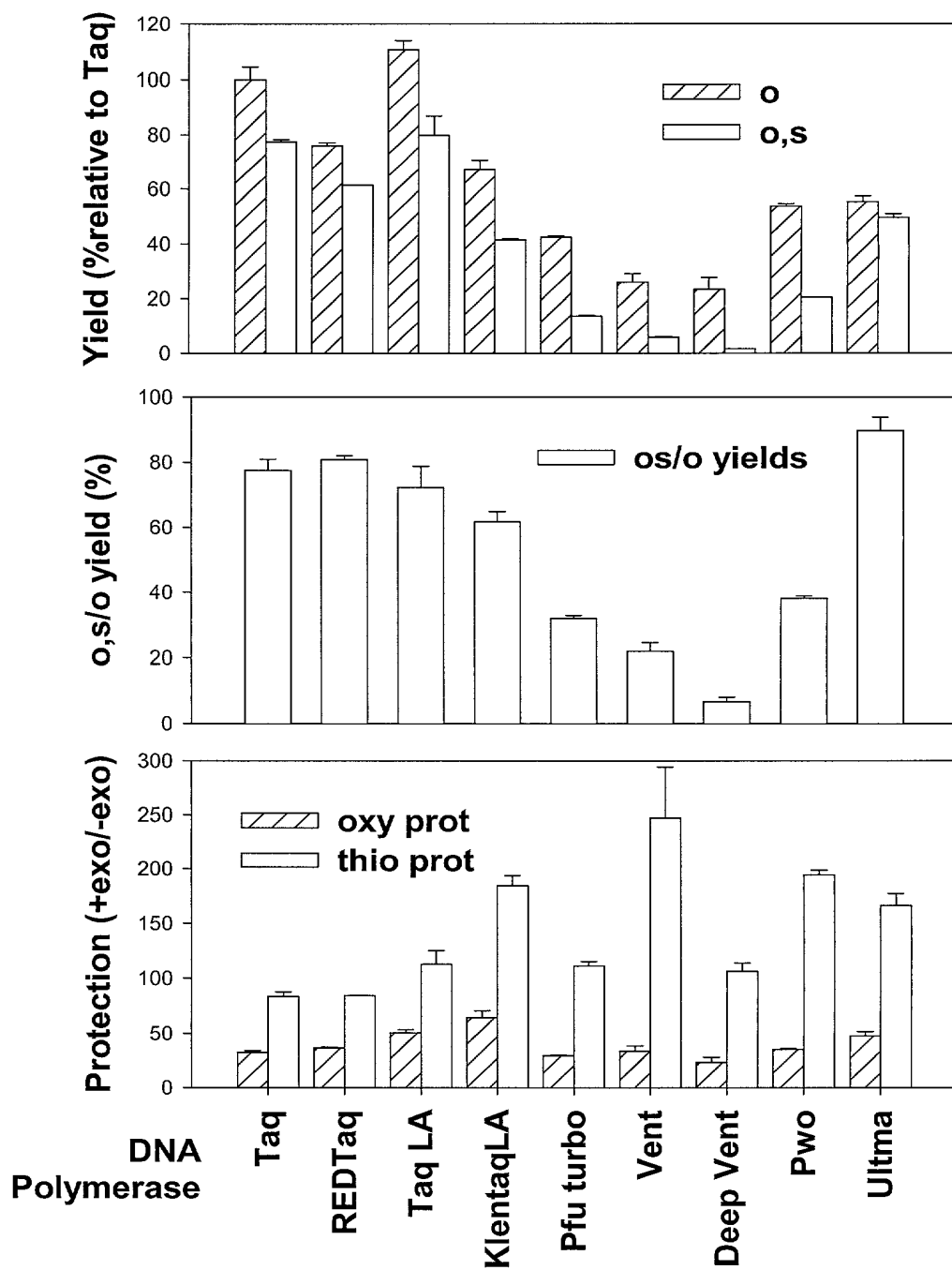


FIG. 3



4/20

FIG. 4

1. Cold PCR, 2. + ExoIII/32PdCTP,
3. TCA precipitate

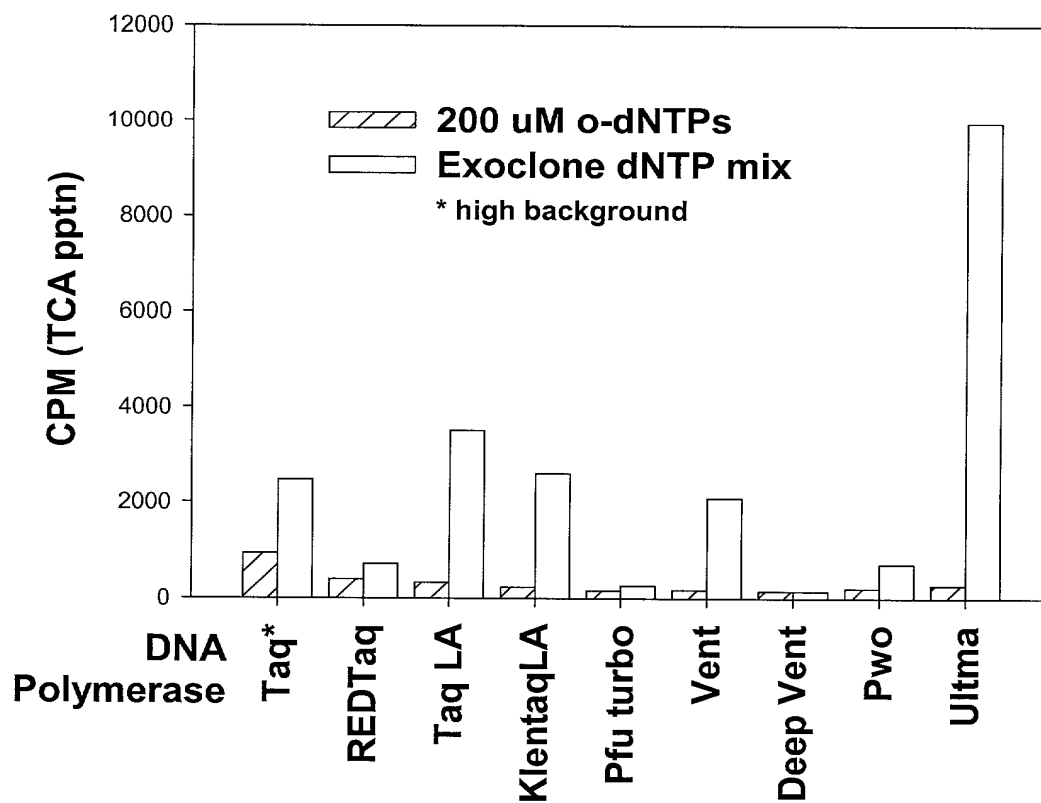


FIG. 5

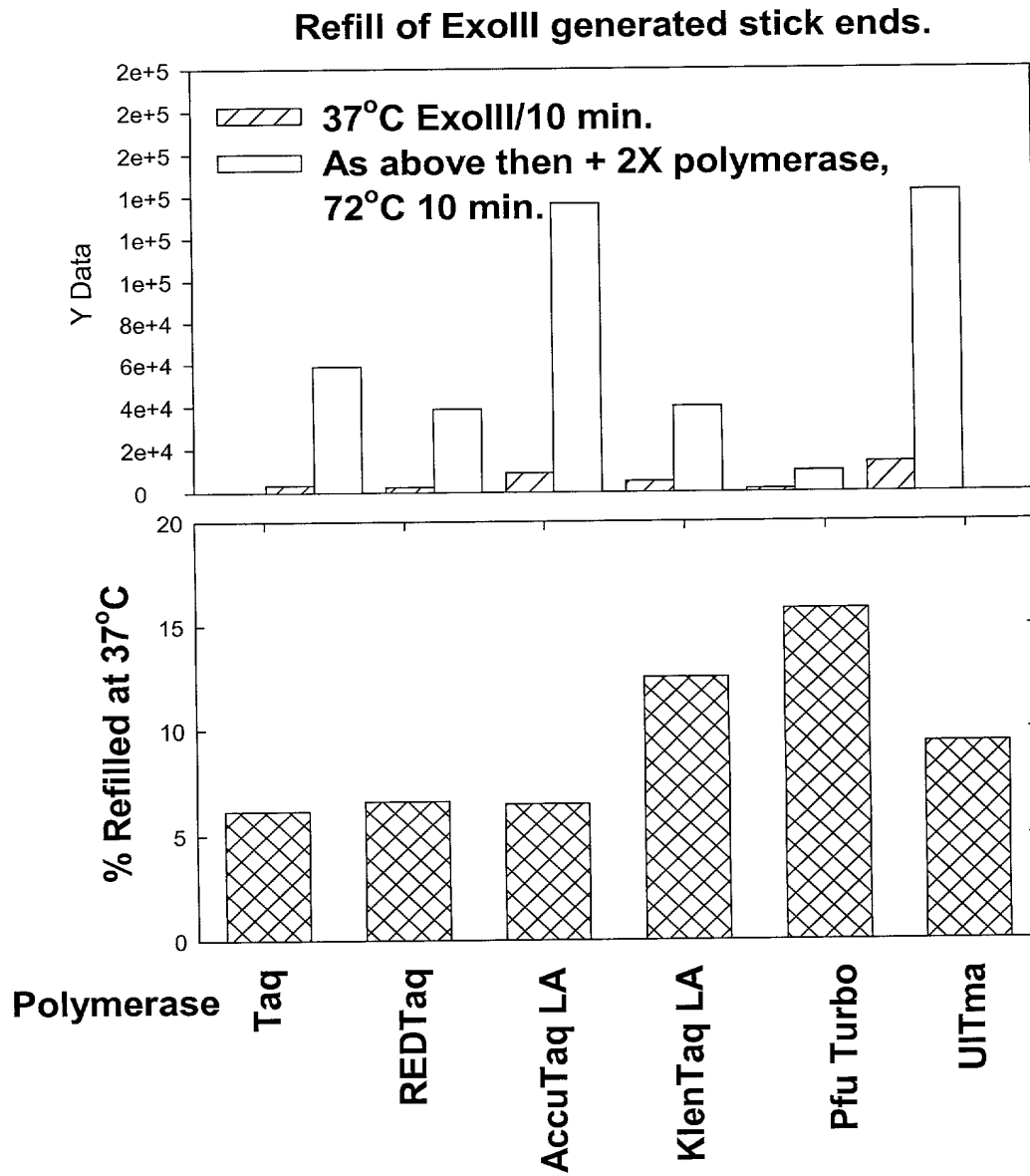


FIG. 6

PCR yield vs. ExoIII protection
 $0.2 \text{ mM} = [\text{o-dNTP}] + [\text{s-dNTP}]$
(quadruplicate data)

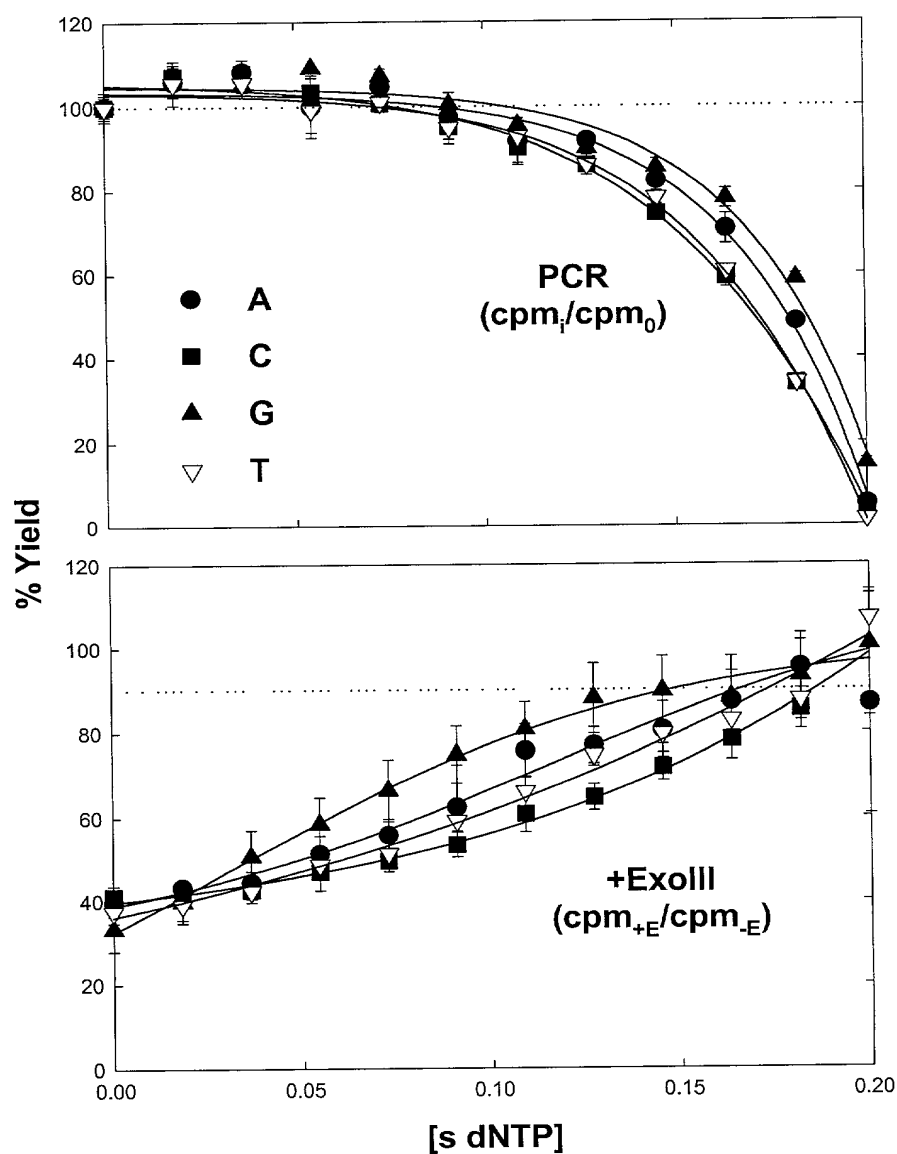
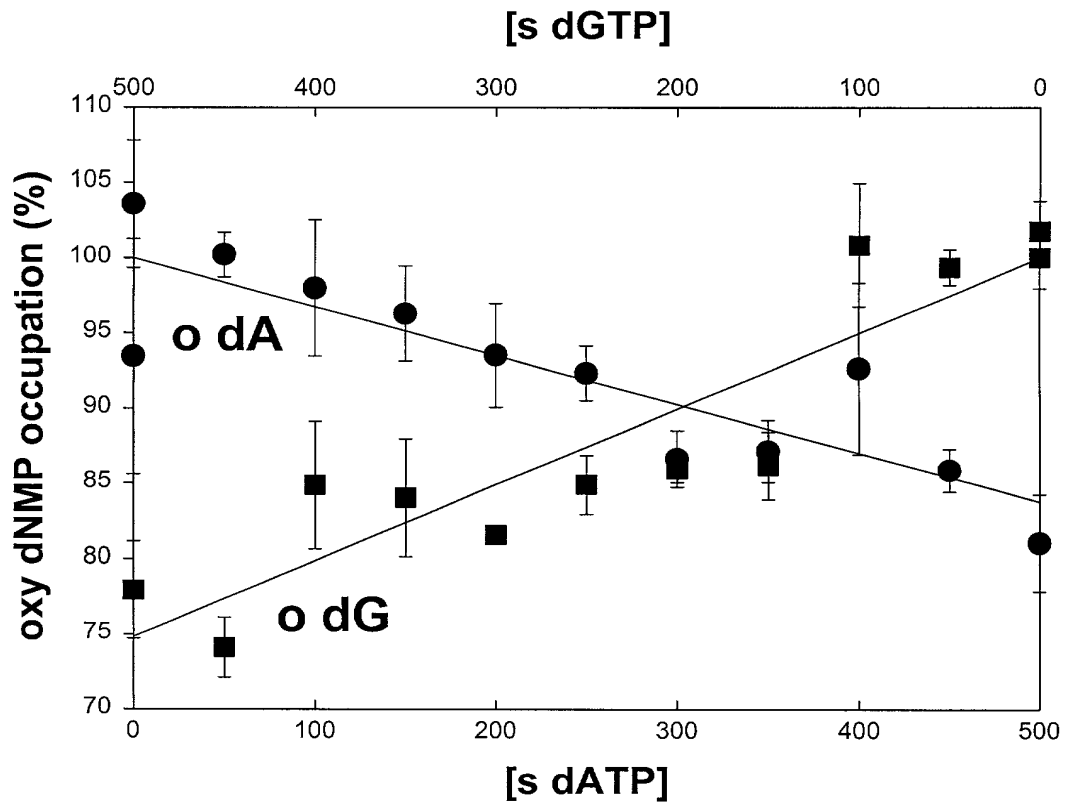


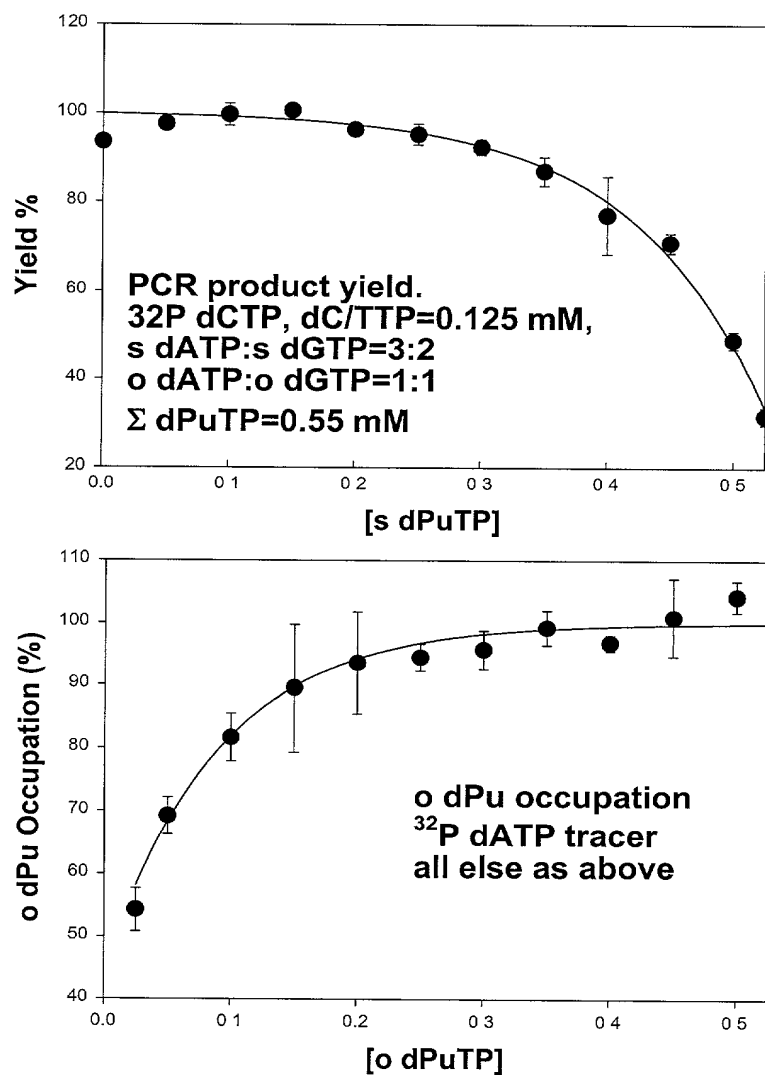
FIG. 7

o dA, dG occupation of PCR product
as a function of [s dA/GTP]
[dC/TTP]=100 μ M, [o dA/GTP]=50 μ M
[s dATP]+[s dGTP]=500 μ M



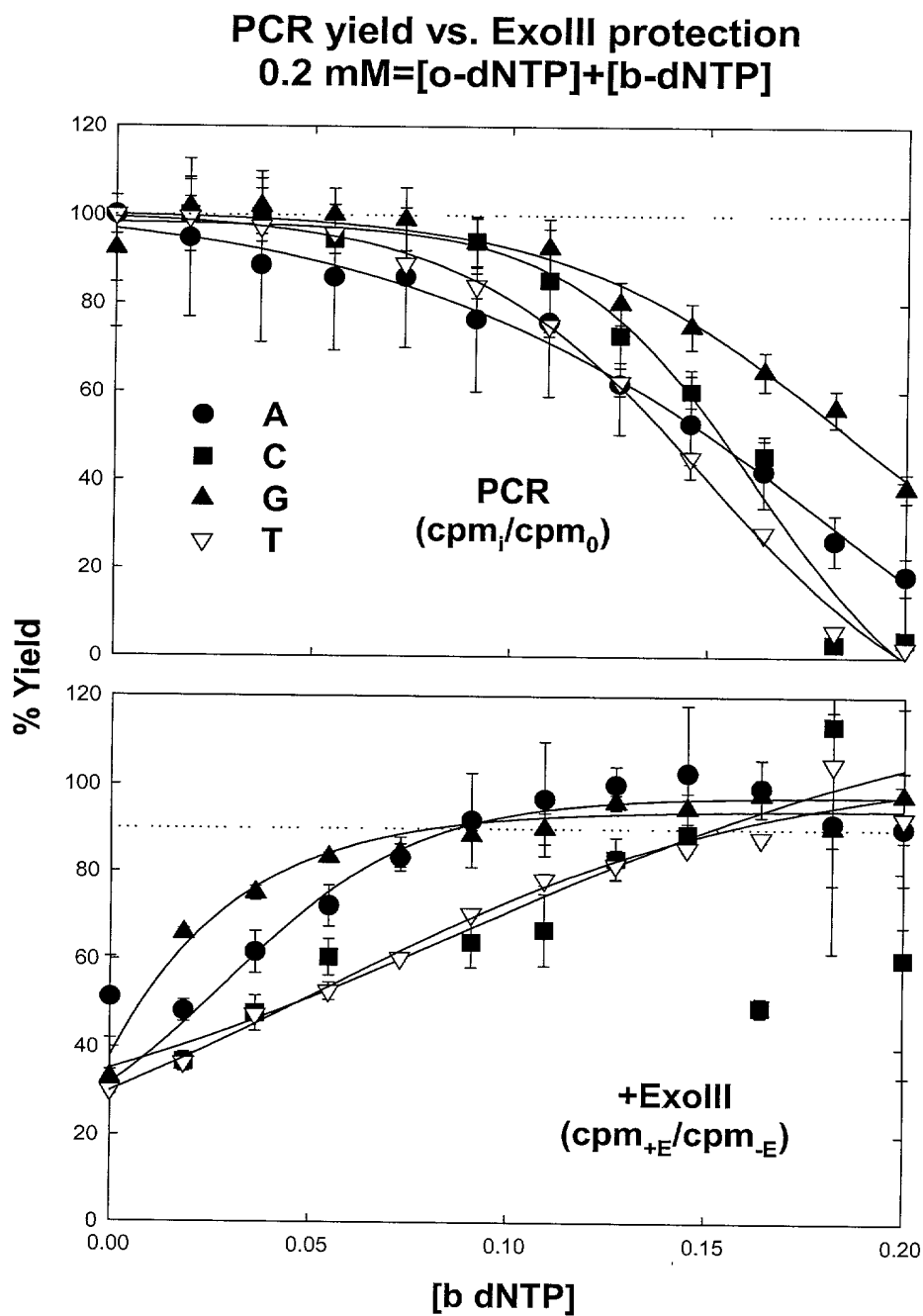
8/20

FIG. 8



9/20

FIG. 9

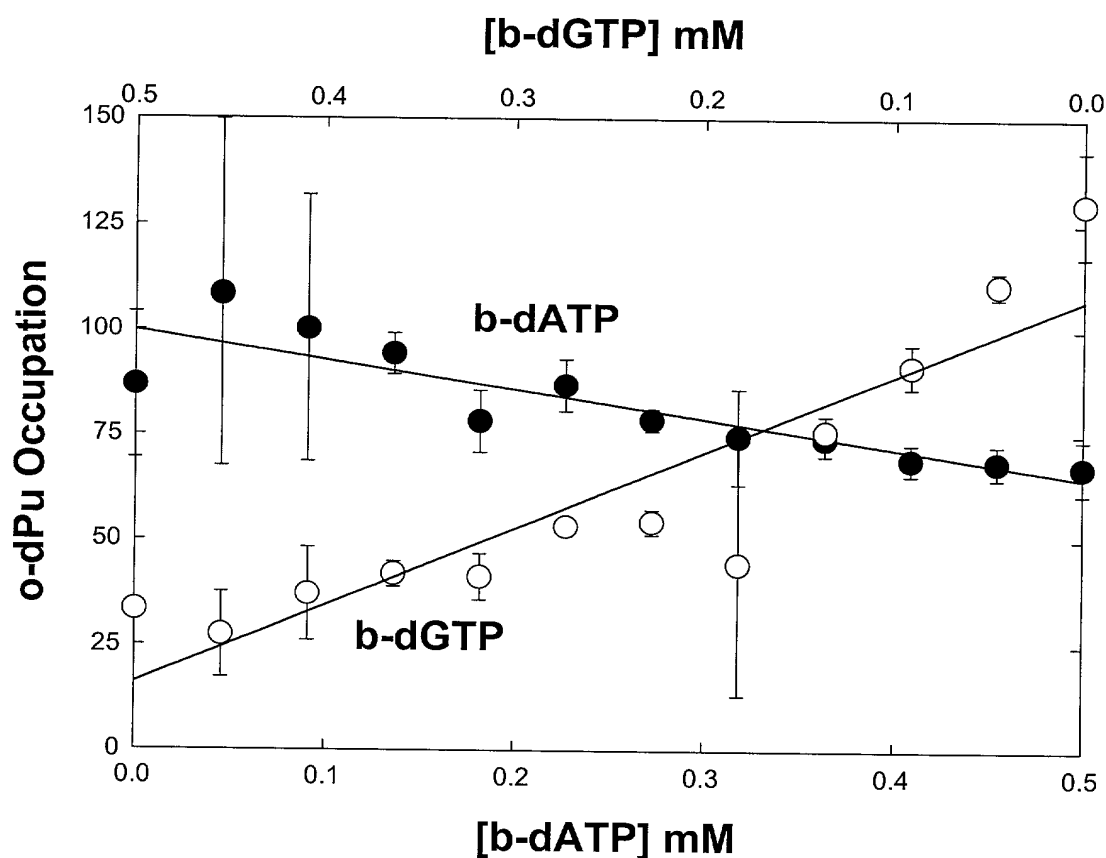


10/20

RECOMBINANT DNA PROCESSES USING A dNTP MIXTURE
CONTAINING MODIFIED NUCLEOTIDES
Ward et al SGM 6938 1

FIG. 10

**o dA, dG occupation of PCR product
as a function of [b dA/GTP]
[dC/TTP]= 0.1 mM, [o dA/GTP]=0.05 mM
[b dATP]+[b dGTP]=0.5 mM**



**o-dA occupation= -70[b-dATP]+99.9
o-dG occupation= -170[b-dGTP]+100
o-dA=o-dG at [b-dATP]/[b-dGTP]=2.5**

11/20

RECOMBINANT DNA PROCESSES USING A dNTP MIXTURE
CONTAINING MODIFIED NUCLEOTIDES
Ward et al SGM 6938 1

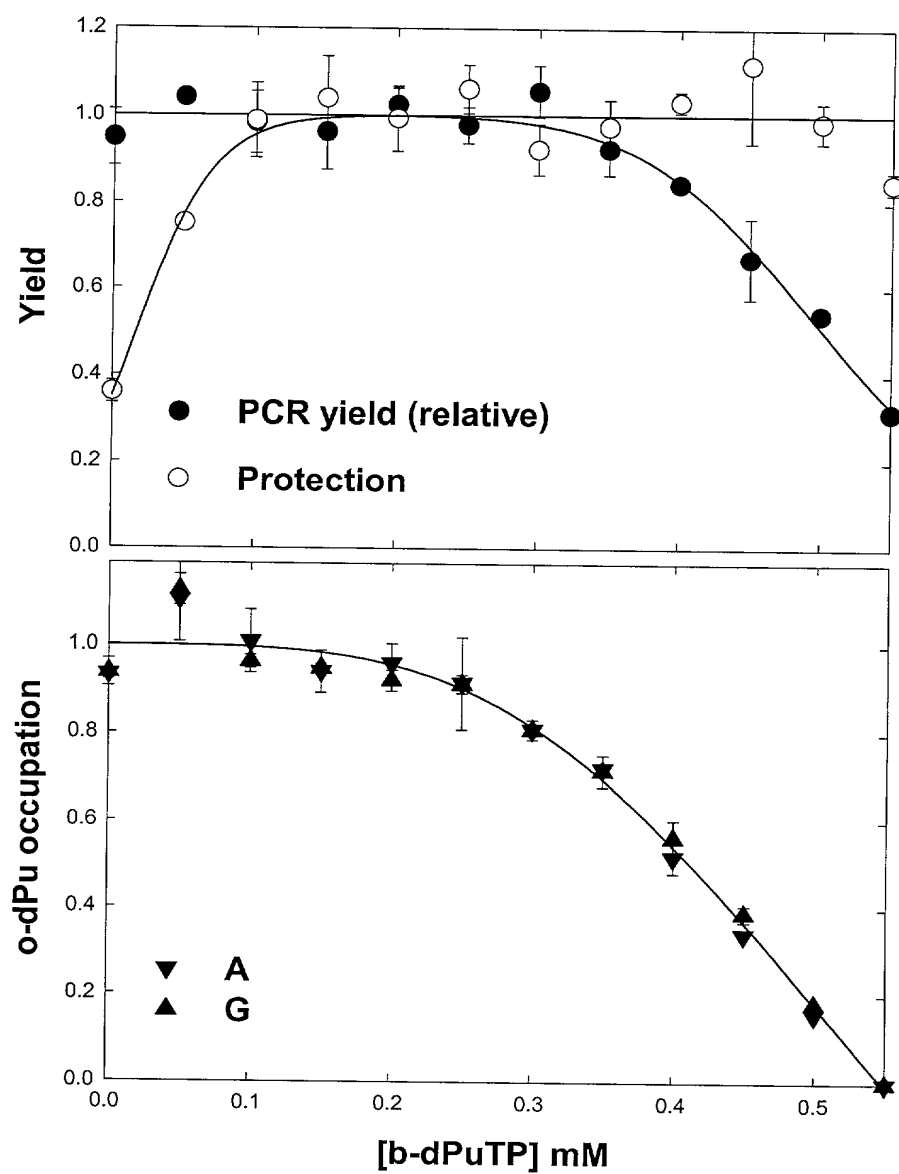
FIG. 11

b-dPuTP vs. o-dPuTP PCR.

b-dATP/b-dGTP=2.5

o-dATP/o-dGTP=1

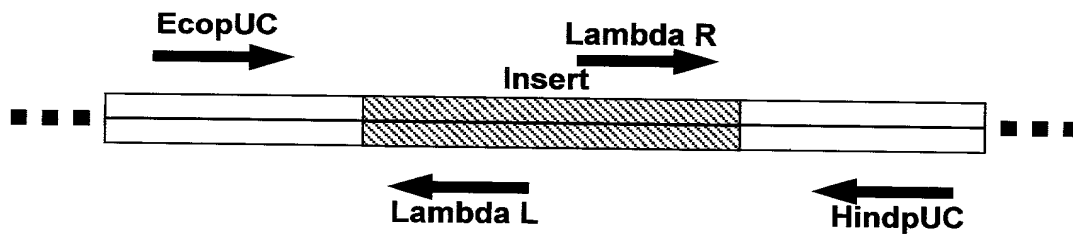
Sum dPuTP=0.55 mM, dPyTP=0.25 mM



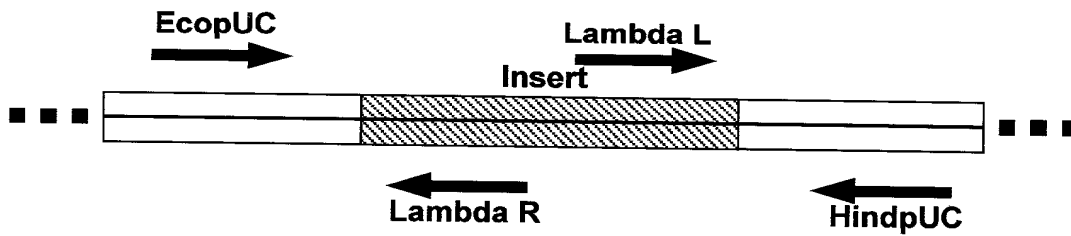
12/20

RECOMBINANT DNA PROCESSES USING A dNTP MIXTURE
CONTAINING MODIFIED NUCLEOTIDES
Ward et al SGM 6938 1

FIG. 12



L-R insertant



R-L insertant

13/20

FIG. 13A

1 2 3 4 5 6

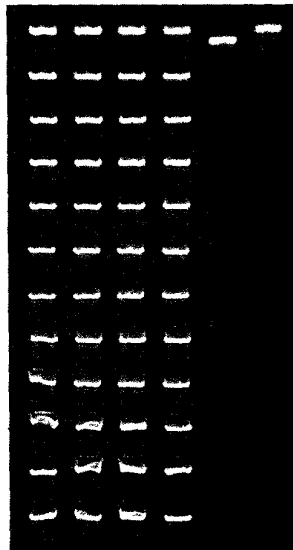
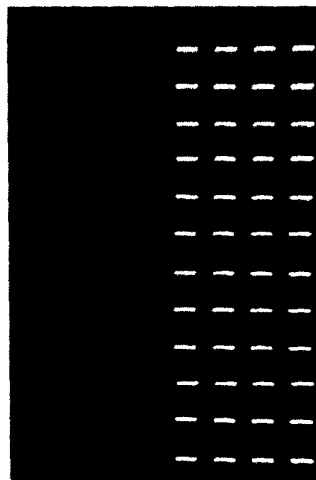


FIG. 13B

HindpUC
Lambda L Lambda R
RE Exo RE Exo

Plasmid Primer
Insert Primer
Cloning Method

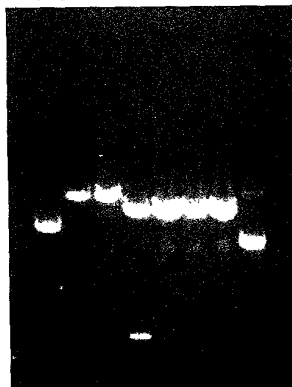


14/20

RECOMBINANT DNA PROCESSES USING A dNTP MIXTURE
CONTAINING MODIFIED NUCLEOTIDES
Ward et al SGM 6938 1

FIG. 14

1 2 3 4 5 6 7 8



1. Uncut pBX
2. BamHI pBX
3. XbaI pBX
4. BamHI/XbaI pBX
5. BamHI pUC19
6. XbaI pUC19
7. BamHI/XbaI pUC19
8. Uncut pUC19

FIG. 15

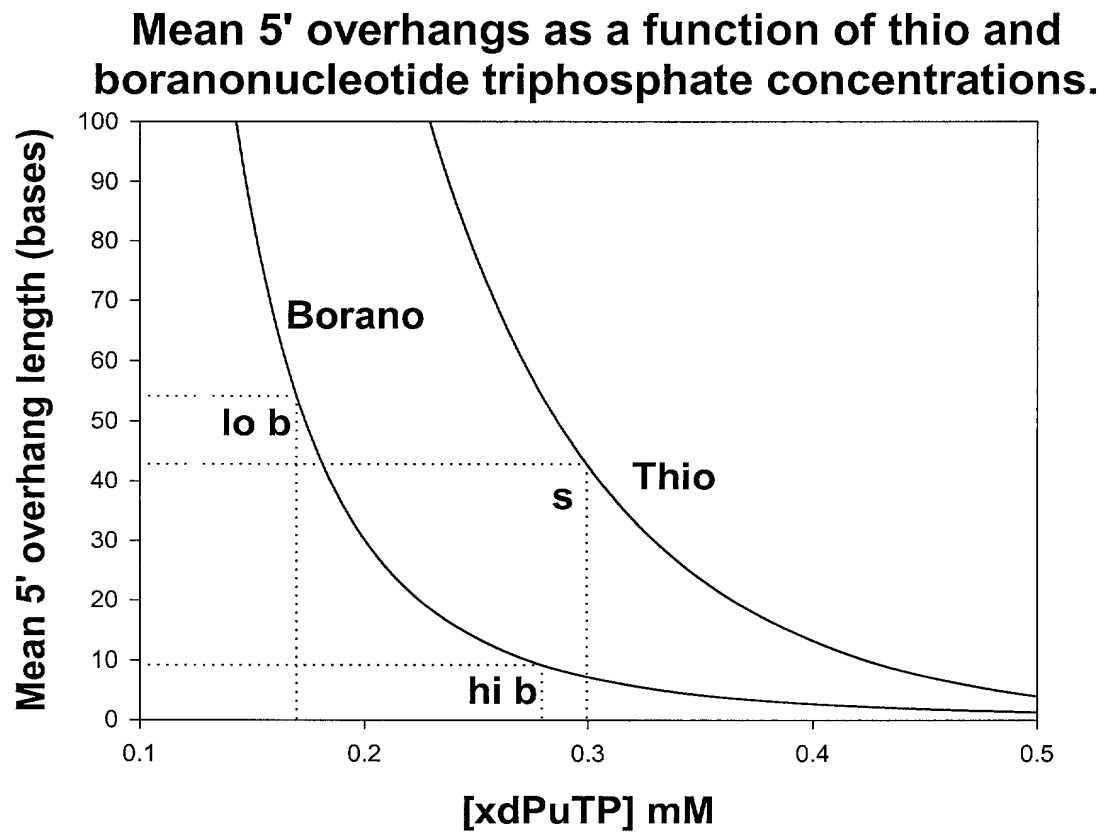
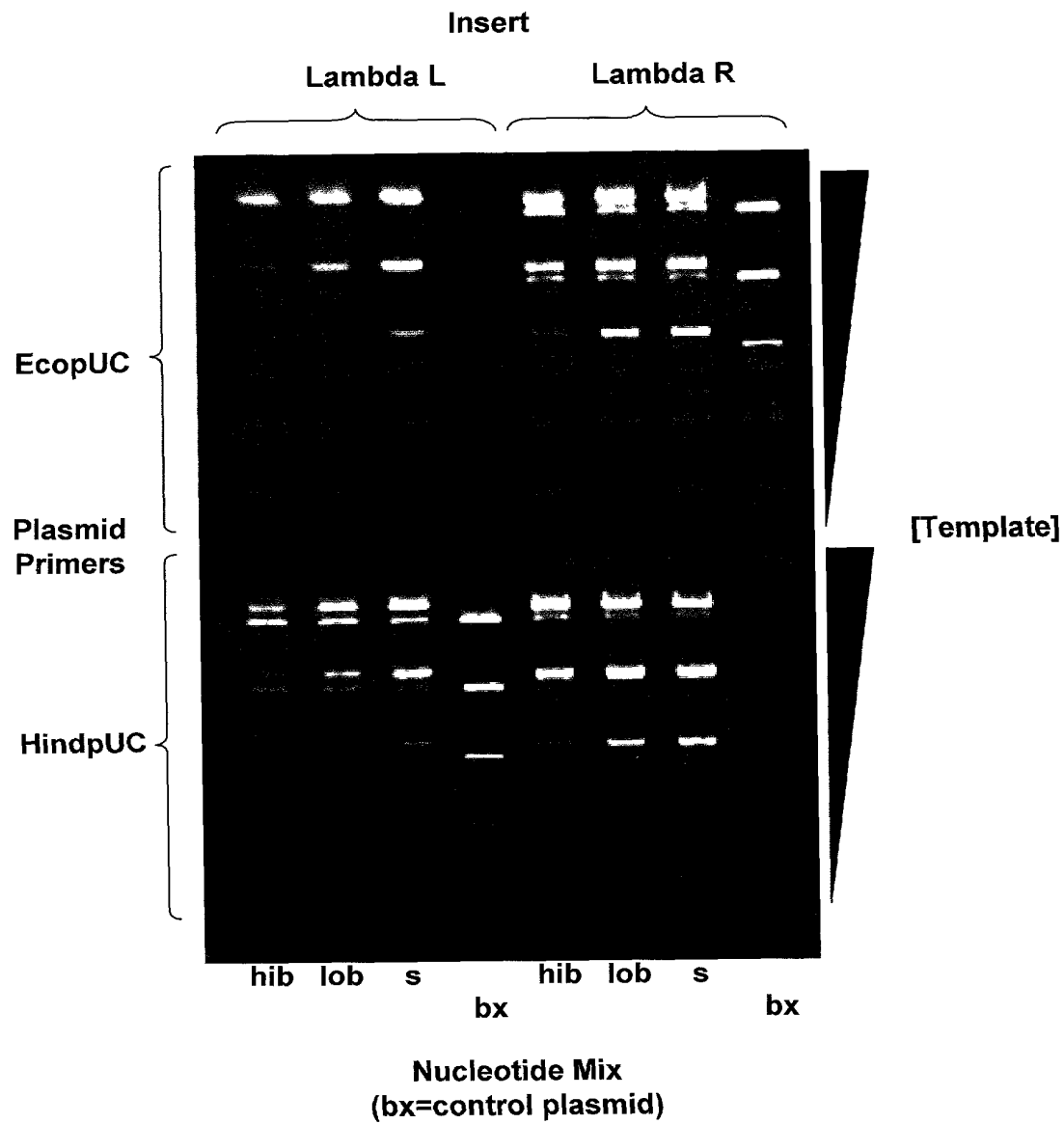
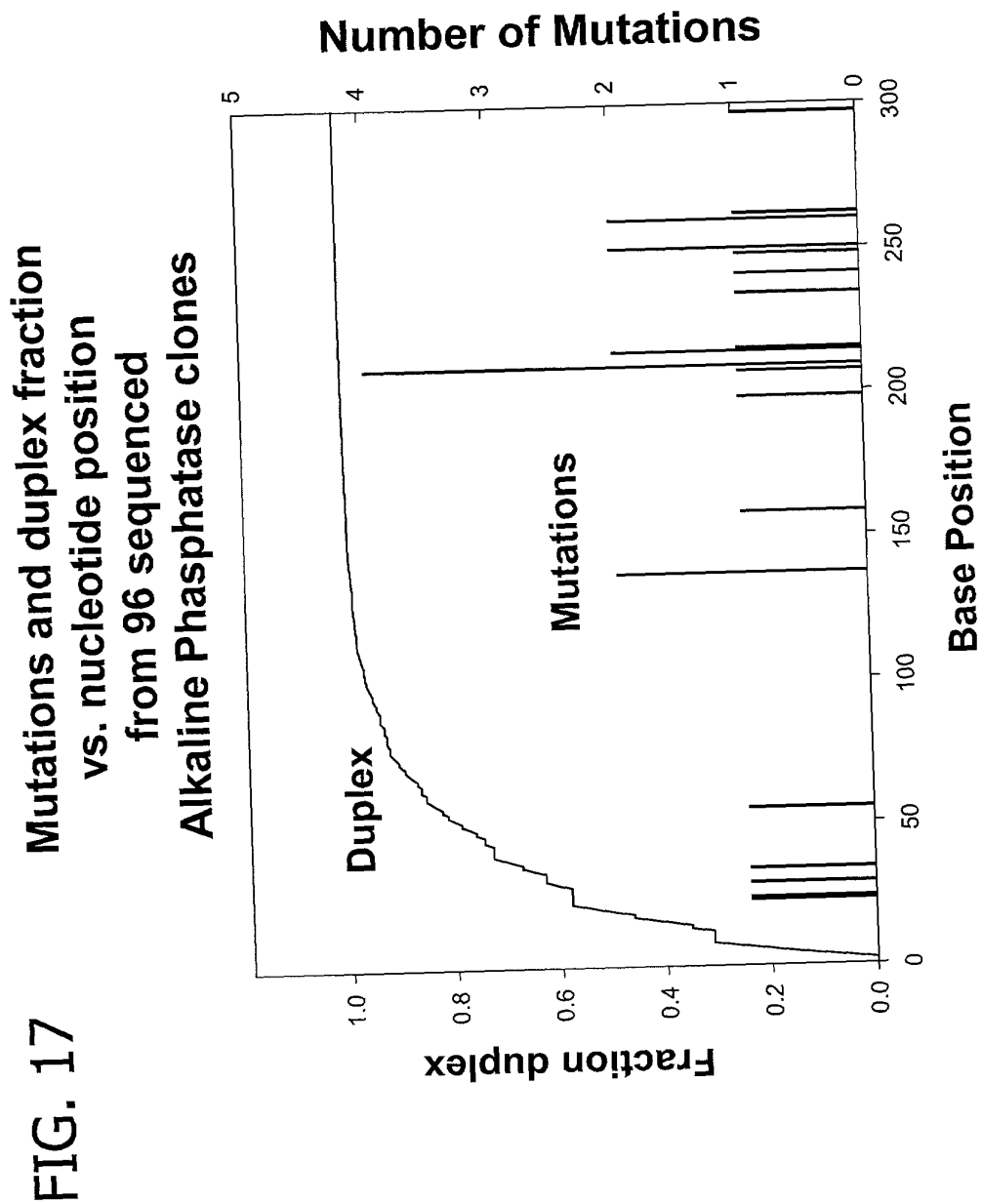


FIG. 16



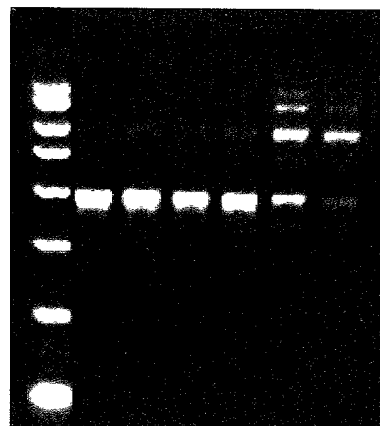


18/20

RECOMBINANT DNA PROCESSES USING A dNTP MIXTURE
CONTAINING MODIFIED NUCLEOTIDES
Ward et al SGM 6938 1

FIG.18

ExoClone
U B E H S X



} Ligation Products
← Digested amplicon

Self Ligation

FIG.19

5' Adaptor

T7 promoter

FLAG Tag

5' GGA TGC TAATACGACTCACTATAG GGAGAAAGGCCACC ATGGACTACAAAGACGATGACGAC A 3' (SEQ ID NO : 11)
 3' CCTACG ATTATGCTGAGTGATATC CCTCTTCCCGGTGG TACCTGATGTTTCTGCTACTGCTG T TCGA 5' (SEQ ID NO : 12)

Forward primer for 2nd PCR

cohesive
end 1

3' Adaptor

cohesive
end 2

GATC T TGA AACTAACCATACGTCAATGTGCCCCACCAGCCTTGTCCTAATA 5' (SEQ ID NO : 13)
 A ACT TTTGATTGGTATGCAGTACACGGGTGGTCTCGGAACAGGATTAT 3' (SEQ ID NO : 14)

Stop
codon

Reverse primer for 2nd PCR

FIG. 20

